STUDIES ON THE INTERACTION OF SOME PLANT PARASITIC NEMATODES ON ECONOMICALLY IMPORTANT PLANTS

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CERTIFICATE

This is to certify that the dissertation entitled "Studies on the interaction of some plant parasitic nematodes on economically important plants" is a faithful record of the bonafide research work carried out by Miss Suhail Anver under my guidance and supervision. Her work is up-to-date and original. She is allowed to submit the dissertation to the Aligarh Muslim University, Aligarh for the consideration of the award of the degree of Master of Philosophy in Botany.

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CONTENTS

INTRODUCTION & REVIEW OF LITERATURE

1. INTRODUCTION & REVIEW OF LITERATURE

A discussion on the importance of plant parasitic nematodes would be more meaningful if the amount of damage done by nematodes to the crop is fully known. Unfortunately the correct and detailed information about the losses caused by nematodes is still scanty. However, some workers have attempted to estimate the magnitude of crop losses due to nematodes in terms of money. For instance, the yearly losses estimated by the United States Department of Agriculture (U.S.D.A.) in 16 crops amount to $ 372,335,000 (Taylor,1967). Stapel (1953) estimated an average annual loss of 50,000,000 Kroner (£ 2½ million) resulting from Heterodera avenae in Denmark. Southey & Samuel (1954) estimated an average annual loss in potato to the tune of £ 2 million resulting from H. rostochiensis in U.K. and Wales in 1949. Some other estimates of average annual losses due to nematodes range from 250 million dollars (Hutchinson et al.,1961) to 500 million dollars (Cairns,1955). Recently Feldmesser et al. (1971) while working on 16 field crops, 23 fruit and nut crops, 24 vegetable crops and all ornamental crops in U.S.A., estimated an average annual loss of $ 1,590,326,934 due to nematodes.

In our country, it is not fully known as to how

much the nematodes cause losses by way of damage to crops. However, Van Berkum & Seshadri (1970) estimated the loss of $ 10 million from 'ear cockle' disease caused by Anguina tritici in wheat and $ 8 million due to 'molya' disease caused by Heterodera avenae in Rajasthan. Moreover, crop losses in coffee caused by Pratylenchus coffeae worth $ 3 million was also assessed.

Plant nematodes, a designation given to those nematodes living pathotrophically on living plants, are significant components of the biotic community of soil. The nematodes are capable of causing important plant diseases independently. In nature, however, they rarely occur in monospecific communities. Rather the nematode communities are dynamic and their members are constantly interacting with each other as well as with other organisms, plants and the environment. Soil is rich in fauna and flora of micro-organisms, so under favourable conditions there may be several kinds of interactions leading to various disease complexes with considerable overlappings. Therefore it is logical to assume that the net result of damage caused to plants by various disease complexes may be different to the one caused by the monopathogenic condition. Further, the nature and behaviour of different disease complexes caused by certain sets of pathogens may not be the same under different ecological conditions.

While feeding on plant tissues the parasitic nematodes cause extensive damage in the form of injuries which sometimes give way to secondary invadors which are otherwise unable to do so alone. Some nematodes are vectors of viruses and bacteria etc. The different associations of nematodes are sometimes capable of breaking the resistance of plants to different pathogens.

There are several basic ways by which two organisms may interact (Odum, 1971):

1. Neutralism - in which neither population is affected by association with each other.
2. Competition-direct inhibition type - in which both populations actively inhibit each other.
3. Competition - resource use type - in which each population adversely affects the other in the struggle for resources in short supply.
4. Ammensalism - in which one population is inhibited and the other is not affected.
5. Parasitism and Predation - in which one population adversely affects the other by direct attack but is nevertheless dependent on the other.
6. Commensalism - in which one population is benefited but the other is not affected.
7. Protocooperation - in which both populations benefit by the association but the relations are not obligatory.
8. Mutualism - in which growth and survival of both populations are benefited and neither can survive under natural conditions without the other.

Competitions - both inhibition and resource use types and ammensalism are designated as 'negative interactions'; commensalism, protocooperation and mutualism as 'positive interactions' and parasitism and predations are 'Positive as well as negative interactions'. Neutralism is ofcourse neutral interaction.

In case of nematodes, Pitcher (1965) suggested the following possibilities in different interactions where the nematodes may act as:

(1) Vectors of pathogens capable of self-establishment once in contact with the host.

(2) Vectors of pathogens incapable of self-establishment unless introduced below the epidermis.

(3) Mechanical wound agents.

(4) Providers of necrotic infection-courts.

(5) Modifier of substrates.

(6) Breakers of disease resistance.

(7) Deterrents of plant disease.

Interactions among species of plant parasitic nematodes can be considered ecological or etiological and the two aspects are interrelated. Ecological interactions affect the reproductive capacity of the populations where as etiological interactions alter the development of plant disease. The relationship between the ecology and etiology of the interaction is based on the correlation of numbers of nematodes with the amount of disease. However because

nematode reproduction is not always related to the susceptibility of the plant to damage, the two aspects of the interaction may behave independently.

The literature on interactions of plant parasitic nematodes with other soil biota is very comprehensive, but in the present treatment the matter of discussion is limited only to the interactions between nematodes. This aspect has also been reviewed by Powell (1971), Norton (1978), and Dropkin (1980).

A review, in detail, on the interactions between plant parasitic nematodes is given below. It is divided into the following categories:

1. Intra-specific interaction
2. Inter-specific interaction
3. Inter-generic interaction
    - i. Interaction between two ecto-parasites
    - ii. Interaction between two migratory endo-parasites
    - iii. Interaction between two sedentary endo-parasites
    - iv. Interaction between ecto-and migratory endo-parasites
    - v. Interaction between ecto-and sedentary endo-parasites
    - vi. Interaction between migratory endo-and sedentary endo-parasites
    - vii. Interaction between ecto-,migratory endo- and sedentary endo-parasites.

I. INTRA-SPECIFIC INTERACTION:

It deals with the interaction between two populations

of the same species. In an interesting study Ismail & Alam (1975a) noted poor larval penetration of Meloidogyne incognita into tomato roots pre-infected with the same species of the nematode. They presumed that the phenomenon of competition was responsible for the inhibition in the subsequent larval penetration or perhaps the already established root-galls produced certain kinds of chemicals, physiological or physical barriers. However, there was a synergistic effect of the sequential inoculation of the same species of the root-knot nematode on the reduction in plant growth and increase in the root-knot development.

INTER-SPECIFIC INTERACTION:

This aspect covers the interaction of two or more nematode species of the same genus. In this type the nematodes have same feeding nature hence the competition is in between them. Limited information is available about the inter-specific interactions of nematodes.

Chapman (1959b) studied the comparative effect of Meloidogyne incognita and M. hapla on the growth of 'Kenland red clover' and 'Atlantic alfalfa'. M. incognita caused more reduction in the growth of red clover and alfalfa in limited time than M. hapla. Chapman (1965) again reported that M. incognita multiplied rapidly at low temperature in the case of combined inoculation.

M. incognita suppressed M. hapla on M. incognita-resistant tobacco cultivars 'NC-95' and 'NC-2512' in green-house experiments at moderate temperatures. In simultaneous inoculations of both species, M. incognita penetrated the roots much faster than M. hapla, and thus all of the infection sites were occupied and destroyed by the hypersensitive reaction (Johnson & Nusbaum, 1970).

Paez et al. (1976) observed that M. incognita and M. javanica together brought about significant growth reduction of tobacco cultivars 'NC-95' and 'Hicks'.

Mahajan & Mangat (1984) studied reaction of some root-knot resistant tomato varieties to a mixed population of M. incognita and M. javanica and found that Bigset, Bonus, Conterra, Better, Boy, Monte-carlo, Beef master, VFN-360, Piernita, Motabo, Motella, Hessoline, CI-3279, CI-3104 and CI-3110 did not exhibit presence of egg masses, females or galls. Variety Beefmaster-850, supported very low population. Rest of the lines showed varying degrees of resistance/susceptibility.

Kinloch & Allen (1972) reported that there was a predominance of M. javanica over M. hapla when present together in tomato. The predominance increased with increasing the mixed species inoculum levels. They also observed that the invasion by M. hapla was more density dependent than M. javanica.

Misaghi *et al.* (1974) observed an antagonistic relationship of *M. incognita*, *M. javanica* and *M. arenaria*.

Ferris *et al.* (1967) reported that *Pratylenchus penetrans* reduced the populations of *Pratylenchus alleni* on soybean when present together.

Positive interactions occurred between *P. minor* and *P. zeae* on several varieties of corn and between *P. minor* and *P. brachyurus* on several soybean cultivars after 50 days in green-house experiments (Johnson & Nusbaum, 1968). *P. zeae* populations were higher with *P. minor* on two varieties of corn while populations of *P. minor* were higher with *P. zeae* on five corn cultivars; *P. brachyurus* stimulated reproduction of *P. minor* on three varieties of soybean.

III. INTER-GENERIC INTERACTION:

These interactions involve two or more genera of varying feeding habits. For convenience the different combinations are discussed separately as under:

1. Interaction between two ecto-parasites:

Ecto-parasites again have different groups according to their parasitic habits. Some ecto-parasites feed only on epidermal cells and root hairs. The association with the host is of short term. Representative genera include

Tylenchorhynchus, Trichodorus, Criconemoides, Helicotylenchus and Hoplolaimus etc.

A more specialized group of ecto-parasites is of those having relatively long stylets. They feed on cells below the epidermis and remain completely outside the root tissue, but the parasitic relationship with the plant is more complex. The group includes Xiphinema, Longidorus, Belonolaimus, Dolichodorus and Hemicycliophora etc.

The shallow epidermal feeders are more antagonistic to each other than the deep feeding nematodes.

Bird & Jenkins (1964) observed that when crane-berry roots were inoculated simultaneously with Hemicycliophora similis and Macroposthonia xenoplax there was an increase in the population of the former. Johnson (1969,1970) while studying on interaction between Criconemoides arnatus, Tylenchorhynchus martini & Belonolaimus longicaudatus on six varieties of Burmuda grass, reported that reduction in the final population of B. longicaudatus was least affected.

Weischer (1974) studied competition between Aphelenchoides ritzemabosi and D. dipsaci on tobacco. He reported that D. dipsaci was inhibited by A. ritzemabosi in simultaneous inoculation on tobacco but A. ritzemabosi was not affected.

The reproduction of Belonolaimus longicaudatus was increased by the presence of Hoplolaimus galeatus in greenhouse experiments after 63 days on cotton (Yang et al.,1976). Prasad & Rao (1977) reported that interaction of Tylenchorhynchus claytoni and Helicotylenchus crenatus on rice resulted in significant decrease of H. crenatus. In concomitant inoculation Helicotylenchus crenatus inhibited population build-up of Tylenchorhynchus dubius on tomato (Krishnapa & Prasad,1979).

Kraus-schmidt & Lewis (1981) observed that in concomitant populations of Hoplolaimus columbus and Scutellonema brachyurum on cotton the reproduction of both the species was mutually stimulated after 90 days.

Combined inoculations of Merlinius brevidens and Tylenchorhynchus vulgaris on wheat indicated that Merlinius brevidens significantly reduced the population of T. vulgaris (Upadhyay & Swarup,1981).

ii. Interaction between two migratory endo-parasites:

Migratory endo-parasites live inside the root tissue but some times moves outside it. Examples of migratory endoparasites are Pratylenchus and Radopholus. The migratory endoparasites are very competitive because they have same feeding site. Moreover the host plays very important role in this interaction.

According to O'Bannon et al. (1976) climatic or edaphic factors may be involved in the interaction between Radopholus similis and P. coffeae which were mutually suppressive on citrus after 10-15 months. There was a definite preference of the nematodes to the soil texture. P. coffeae predominated in fine textured soil while R. similis had the advantage in coarse textured soils.

Acosta & Ayala (1976) reported that P. coffeae antagonized populations of Scutellonema bradys on guinea-yam (Dioscorea rotunda).

Cuarezma Teran & Trevathan (1985) observed that the population densities of Pratylenchus zeae and Quinisulcius acutus on sorghum 6 weeks after inoculation were significantly less in combined inoculations as compared to their separate inoculations.

iii. Interaction between two sedentory endo-parasites:

Sedentary endo-parasites live in the host and have a complex relationship. These are highly specialized parasites. Sedentary endo-parasitic spp. are generally mutually suppressive and cause physiological alterations.

Interaction between cyst and root-knot nematodes is density dependent. Ross (1959,1964) reported that the low populations of M. incognita had no effect on

Heterodera glycines while high population suppressed H. glycines early in the season and stimulated them late in the season.

Jatala & Jensen (1976a) reported double inoculation of M. hapla on sugarbeet had little effect on each other, where as Heterodera schachtii inoculations interacted positively. In double inoculation 10 days apart, there was a 3 to 5 fold increase in the number of infections as compared to a single inoculation of equal numbers of infective juveniles.

Interaction between sedentary endoparasites is also greatly influenced by the time of inoculation. Jatala & Jensen (1972) observed that when Heterodera schachtii preceded M. hapla there was a marked reduction in root-galling but when M. hapla preceded H. schachtii an increase in the cyst formation was noticed. Simultaneous inoculation showed no significant changes in the population of the two nematodes. In a similar study they (Jatala & Jensen,1976b, 1983) found that prior inoculation of H. schachtii in the green house suppressed M. hapla on sugarbeet but previous infection by M. hapla stimulated population of H. schachtii.

Sharma & Sethi (1975) reported that in concomitant inoculation of cowpea with Heterodera cajani and Meloidogyne incognita, the populations of both the nematodes were

suppressed, while the plant growth remain unaffected. In another study Sharma & Sethi (1976) observed that the M. incognita and H. cajani were mutually inhibitory to each other; H. cajani larvae penetrated faster and in higher numbers than M. incognita larvae. They (Sharma & Sethi, 1978) further observed in a green house experiment that prior inoculation of M. incognita was detrimental to Heterodera cajani on cowpea and previous infection by H. cajani was suppressive to M. incognita.

Under green house conditions M. graminicola was found antagonistic to Heterodera oryzicola on rice after 52 days in simultaneous or spatial inoculation of 7 days (Rao & Prassad,1981).

Griffin & Wait (1982) reported a synergistic relationship between Heterodera schachtii and M. hapla. This combination significantly reduced tomato root weight. Inserra et al. (1984) observed that neither Heterodera schachtii nor M. hapla had an adverse effect on the Pf/Pi ratio of Nacobbus aberrans. N. aberrans is consiuered to be the less aggressive on sugarbeet than either of H. Schachtii or M. hapla. Griffin (1985) reported that M. hapla was adversely affected by Heterodera schachtii in combined inoculations of the two nematode Spp. on tomato.

In a histopathological study of maize root, Kaul &

Sethi (1982) found that H. zeae penetrated at both meristematic and zone of elongation while M. incognita penetrated at meristematic zone. In case of simultaneous inoculation the penetration of M. incognita was adversely affected by the presence of H. zeae. This condition was, however, not found in the reverse course.

Interaction between Meloidogyne and Rotylenchulus spp. can be suppressive for either or both the Spp. Singh (1976) reported that in the combined infection of R. reniformis and M. incognita on soybean, the population of R. reniformis was more adversely affected but there was no effect on M. incognita. In a similar investigation Mishra & Gaur (1981) studied the effect of individual and combined inoculation of M. incognita and R. reniformis on the growth of black gram (Vigna mungo). In concomitant inoculations the extent of growth reduction was relatively less than the individual effect. They again reported (Mishra & Gaur, 1981) that M. incognita and R. reniformis affect the plant growth of 'Moth' bean, Vigna aconitifolius which was negatively correlated to the level of inoculation of both the nematodes. M. incognita and R. reniformis were found mutually antagonistic on grape seedlings, suppressing the population of each other, with increasing levels of their respective inoculum (Ras & Seshadri, 1981). The suppressive effect by M. incognita was more on R. reniformis.

Interaction between Meloidogyne and R. reniformis depends on density and time as reported by Ras & Prasad (1971). In 60 days R. reniformis increase more rapidly and suppressed M. javanica but no differences occurred after 90 days. Taha & Kassab (1980) noted that simultaneous inoculation of cowpea with M. javanica and R. reniformis resulted in the prior invasion by M. javanica but less increase in its population in comparison with R. reniformis then when M. javanica was present alone. In a comprehensive study Thomas & Clark (1980,1981,1983) noted that low levels of R. reniformis inhibited M. incognita on sweet potato where as M. incognita had no effect on R. reniformis. High levels of M. incognita however suppressed R. reniformis while R. reniformis had no effect on the root-knot nematode.

Taha & Sultan (1977) observed that the populations of Rotylenchulus reniformis and Tylenchulus semipenetrans were mutually suppressive in mixed infestation on grape wine.

iv. Interaction between ecto- and migratory endo-parasites:

Interestingly these interactions are antagonistic. Here the host has a key role in most of the cases.

Chapman (1959a) noticed that there was no significant difference in the plant growth of red clover and alfalfa when inoculated concomitantly or separately with Pratylenchus penetrans and Tylenchorhynchus martini.

Populations of T. martini was reduced in case of combined inoculations.

Miller & Mc-Intyre (1975) and Mc-Intyre & Miller (1976) observed that simultaneous inoculations of Pratylenchus penetrans and Tylenchorhynchus claytoni prevented the entry of P. penetrans in tobacco roots.

According to Amous & Taylor (1974) the nematode-nematode interactions may be time dependent. They provided evidence that Tylenchorhynchus agri suppressed the reproduction of P. penetrans on red clover after 3 months in green-house experiment but not after 5 or 7 months.

Similarly in the simultaneous inoculation of Pratylenchus scribneri and Scutellonema brachyurum on Amaryllis, Nong & Weber (1965) noted that after two months of inoculation, no foliar symptoms were apparent, but after 4 months most of the roots were destroyed, bulbs were infected and foliage was reduced.

Acosta & Ayala (1976) observed that simultaneous inoculation of P. coffeae and Scutellonema bradys suppressed more top growth and storage food quality of Guinea-Yam (Dioscorea rotundata) but when they were inoculated separately there was less suppression.

Combined inoculations of Pratylenchus

neoamblycephalus and Criconemoides xenoplax on "Myrobalam" plum caused darkening of roots and reduction in feeder roots (Braun et al.,1975).

Pinochet et al. (1976) reported that concomitant inoculations of Pratylenchus vulnus and Xiphinema index caused greater growth reduction of grape wine (Vitis vinifera) seedlings of variety "Thompson seedless". Moreover the population of X. index was reduced in the presence of Pratylenchus vulnus.

Siyanand et al. (1982) investigated the life cycles of Tylenchorhynchus vulgaris, Pratylenchus thornei and Hoplolaimus indicus individually and in combined infestation in maize and observed that the life cycle of T. vulgaris was shortened by 13-15 days in combined inoculation. However, no such differences were observed in the case of P. thornei and H. indicus.

McGawley & Chapman (1976,1983) were of the opinion that host suitability affects the interaction between ecto-parasites and migratory endo-parasites. Populations of Pratylenchus projectus were suppressed by Helicotylenchus pseudorobustus and criconemella similis on soybean in a green-house test after 50 days. Soybean was a good host for C. similis, a moderately good host for H. pseudorobustus and a poor host for P. projectus.

v. Interaction between ecto- and sedentary endo-parasites:

Ecto-parasitic and sedentary endo-parasitic nematodes have quite different feeding sites. So these nematodes can live in the same host without influencing each other or they may be mutually detrimental or stimulatory.

Alam et al. (1975) reported that combined inoculation of M. incognita and Tylenchorhynchus brassicae caused significantly more reduction in the growth of tomato plants than caused by the single pathogen. When inoculated simultaneously with M. incognita, the population of T. brassicae decreased to levels below the initial inoculum. T. brassicae, on the other hand, did not materially affect the development of the root-knot nematodes. In a similar study Khan et al. (1978) found that when M. incognita and T. brassicae were present together on tomato their multiplication rate was adversely affected. The reduction in the multiplication rate of M. incognita in the presence of T. brassicae was more than that of T. brassicae in the presence of M. incognita.

Sikora et al. (1972) found that T. agri inhibited the population of M. naasi whereas the presence of M. naasi did not affect the population of T. agri.

Combined effect of M. incognita and T. vulgaris on bajra plant was additive though T. vulgaris exhibited antagonistic relationship with M. incognita population (Vaishnav & Sethi,1978).

Hasan (1985) studied the interrelationship between Meloidogyne incognita and Merlinius brevidens in the green house by inoculating tomato seedlings simultaneously and sequentially with the nematode at two inoculum levels. One species reduced the other significantly in treatments where one numerically dominated over the other or where both of them were present at the same highest inoculum level in simultaneous or sequential inoculation treatments. The size of adult females of M. incognita and the giant cells was significantly reduced while the male/female ratio of both the nematodes increased significantly both in the simultaneous and in the sequential inoculations.

Kaul & Sethi (1982) noticed that Meloidogyne incognita was adversely affected by the presence of Heterodera zeae and Tylenchorhynchus vulgaris on Maize. Population of T. vulgaris was also reduced by H. zeae and M. incognita. But Tylenchorhynchus vulgaris finally enhanced the penetration of M. incognita.

Hoplolaimus columbus antogonized M. incognita on cotton probably by altering the physiology of the host

rather than by competing for feeding sites (Kraus et al., 1972). According to Bird et al. (1974) Hoplolaimus columbus and M. incognita significantly inhibited the population of concomitant species on cotton.

Hasan & Alam (1975) reported that the competition of M. incognita and Hoplolaimus indicus is responsible in the inhibition of H. indicus or it may also be possible that the root-knot infection brought about certain physico-chemical changes in the host root which were responsible for the reduction in the population of H. indicus on tomato.

Misra & Das (1977) observed that three ectoparasitic nematodes, e.g. Criconemoides arnatus, Hoplolaimus indicus, Tylenchorhynchus nudus in combination significantly reduced the infectivity of Meloidogyne incognita in brinjal. In this operation dry weight of shoot and root was also affected.

Kraus-Schmidit & Lewis (1981) inoculated the cotton seedlings with Scutellonema brachyurus, Hoplolaimus columbus and M. incognita singly and in different possible combinations They noted that the population of S. brachyurus increased in the presence of Hoplolaimus columbus but were suppressed by M. incognita, while simultaneous inoculation of H.columbus with either M. incognita or S. brachyurus increased

H. columbus population in cotton.

Yang et al. (1976) studied the interaction between M. incognita, Belonolaimus longicaudatus and Hoplolaimus galeatus and reported that population of M. incognita and Hoplolaimus galeatus were reduced in combined infection.

Malek & Jenkins (1964) reported that concomitant inoculations of Meloidogyne hapla, Criconemoides curvatum and Trichodorus christiei resulted in the population decrease of these nematodes. M. hapla antagonized Xiphinema americanum on alfalfa (Norton, 1969).

According to Santo & Bolander (1977) Macroposthonia xenoplax suppressed M. hapla on concord grape.

Globodera tabacum inhibited Tylenchorhynchus claytoni possibly because the cyst nematode was more persistant (Miller & Wilhelm, 1968).

vi. Interaction between migratory endo- and sedentary endo-parasites:

The migratory endoparasites move from one place to another through root tissues which are ruptured by the sedentary endoparasitic nematodes for the feeding.

According to Graham et al. (1964) Pratylenchus spp. dominated over M. incognita on tobacco. In another report

Pratylenchus was found to have greatly suppressed Meloidogyne spp. on pine apple (Guerount,1968). Johnson & Nusbaum (1970) reported that mixed inoculation of M. incognita and P. brachyurus resulted in a significant increase in population of P. brachyurus on a resistant variety of tobacco.

In a study it was found that the sedentary endo-parasite may stimulate or suppress root penetration by the migratory species depending on suitability of the host. In tomato, which is a better host for M. incognita than P. brachyurus, prior inoculation of M. incognita suppressed penetration by P. brachyurus; however in cotton, which is a good host for P. brachyurus and a poor host for M. incognita, prior infection by M. incognita slightly stimulated penetration by P. brachyurus (Gay & Bird,1973).

Estores & Chen (1970) noticed that penetration of Pratylenchus penetrans was not affected by the presence of Meloidogyne incognita on tomato but the reproduction of P. penetrans was inhibited. However, M. incognita alone caused more severe stunting than caused by their association. In another study they (Estores & Chen,1972) observed that interaction of M. incognita and P. penetrans resulted in the suppression of population densities of both the nematodes when they coinhabited the tomato roots. M. javanica was inhibited by P. minor on tomato (Van Gundy, 1975).

Turner & Chapman (1971) observed that M. incognita and P. penetrans penetrated into the seedlings of "Buffalo" alfalfa and 'Kenland' red clover when inoculatedsingly during 24-27 hr. of incubation at 24°C; simultaneous inoculation did not affect the penetration of either species. They (Chapman & Turner,1972) again found concomitant behaviour of these two parasitic nematodes on alfalfa. The larvae of M. incognita and P. penetrans when inoculated in different combinations, it was observed that the P. penetrans invaded equally well in all the treatments, but P. penetrans deposited 37% fever eggs when low numbers of M. incognita were present. Chapman & Turner (1975) reported 57% fever eggs when high numbers of root-knot nematode were present.

Sikora et al. (1972) also reported that the interaction is often time dependent. P.penetrans initially inhibited M. naasi on creeping bent grass but after ten months had no effect. Amosu & Taylor (1975) reported that after 3 months M. incognita had no effect on population of P. penetrans but suppressed it after 5 months.

When Heterodera trifolii and Pratylenchus penetrans were introduced simultaneously there was an increase in penetration (Freckman & Chapman,1972).

Prior infection by Ditylenchus dipsaci on a

root-knot resistant cultivar of alfalfa reduced the resistance to M. hapla (Griffin,1972). Griffin (1980) again observed that the prior inoculation of seedlings of alfalfa "Vernal-298" (resistant to M. hapla but susceptible to D. dipsaci) with D. dipsaci increased the galling caused by M. hapla and prior inoculation of "Lahonton" (resistant to D. dipsaci but susceptible to M. hapla) with M. hapla increased its susceptibility to D. dipsaci. A combination of M. hapla and D. dipsaci resulted in a synergistic weight depression on "Ranger" at all soil temperatures. On "Nevada" (resistant to M. hapla and D. dipsaci) M. hapla and D. dipsaci failed to break the resistance. Griffin (1981) reported that single and combined inoculation of Heterodera schachtii and Ditylenchus dipsaci significantly reduced root growth of sugarbeet.

Sheela & Venkiteson (1981) reported that Radopholus similis and M. incognita were mutually suppressive on black pepper (Piper nigrum) in green-house experiments.

vii. Interaction between ecto-, migratory endo- and sedentary endo-parasites:

Miller & Wihrheim (1968) observed that multiple inoculation of tobacco with Globodera tabacum, Pratylenchus penetrans and Tylenchorhynchus spp. caused reduction in the infection and survival of the three nematodes.

Sikora et al. (1972) reported that combined inoculation of Meloidogyne naasi with Pratylenchus penetrans and Tylenchorhynchus agri affected the growth of creeping bentgrass, T. agri reduced the population of M. naasi.

M. hapla alone or in combination with Pratylenchus penetrans and/or Tylenchorhynchus agri was highly pathogenic to red clover and also affected the nodulation (Amosu & Taylor, 1974). At high inoculum level of P. penetrans alone with T. agri significantly reduced the gall index of M. hapla.

PLAN OF WORK

Preliminary survey conducted in vegetable fields around the university campus revealed the presence of the root-knot nematode, Meloidogyne incognita and the reniform nematode, Rotylenchulus reniformis in moderate to high population densities around tomato and okra. Some fields harboured both the Spp. Therefore it was considered interesting to study the interrelationships, if any, of these nematodes in relation to plant growth and water absorption capability of roots of tomato and okra. The following experiments were conducted, the results of which embodies the present dissertation:

1. Effect of the root-knot nematode, Meloidogyne incognita

and the reniform nematode, Rotylenchulus reniformis singly or concomitantly on the plant growth, root-knot development and their final populations on tomato cv. Pusa Ruby.

2. Effect of the root-knot nematode, Meloidogyne incognita and the reniform nematode, Rotylenchulus reniformis singly or concomitantly on the water absorption capability of roots of tomato cv. Pusa Ruby.
3. Effect of the root-knot nematode, Meloidogyne incognita and the reniform nematode, Rotylenchulus reniformis singly or concomitantly on the plant growth, root-knot development and their final populations on okra cv. Pusa Sawani.
4. Effect of the root-knot nematode, Meloidogyne incognita and the reniform nematode, Rotylenchulus reniformis singly or concomitantly on the water absorption capability of roots of okra cv. Pusa sawani.

MATERIALS & METHODS

2. MATERIALS & METHODS

2.1. Selection of test materials:

Two nematode species, viz.,the root-knot nematode, Meloidogyne incognita (Kofoid & White) Chitwood, and the reniform nematode, Rotylenchulus reniformis Linford & Oliveira, were selected as test pathogens for the studies. Tomato, Lycopersicon lycopersicum L. cv. 'Pusa Ruby' and okra, Abelmoschus esculentus Moench. cv. 'Pusa Sawani' were used as test plants.

2.2. Preparation of inoculum of the nematodes:

The nematode cultures were maintained on tomato in microplots.

In the case of the root-knot nematode, eggmasses were collected from the infected roots of tomato with the help of sterilized forcep and placed on small coarse seive (1 mm pore size) fitted with moist tissue paper and placed in petridishes (10 cm diameter) containing sterilized water. Second stage juveniles, which were hatched out, were collected along with water from petridishes after every 24 hrs. Fresh water was added to the petridishes each time after withdrawing the nematode suspension. This process was repeated upto 5-7 days. These second-stage juveniles served as the inoculum of the root-knot nematode.

For the extraction of the reniform nematode, soil was collected from around the roots of tomato plants growing in microplots. The soil was processed for extraction of nematodes by using the Cobb's sieving and decanting method along with the modified Bearmann funnel technique (Southey, 1970). The nematode suspension collected from the funnels served as inoculum of the reniform nematode.

Separate water suspensions of the nematodes were gently stirred for making homogenous distribution of nematodes and then 5 ml suspension transferred to the counting dish (Southey,1970) and the nematodes in each sample were counted under stereoscopic microscope. An average of five counts were made in each case to determine the density of nematodes per unit volume of the suspension.

2.3. Maintenance of test plants:

Sandy loam soil, which is commonly found in Aligarh, was collected from a fellow field and further passed through a coarse seive (1 mm pore size) to remove stone particles and debris etc. Compost manure at the rate of 1 g N/kg soil was then added and thoroughly mixed with the soil. Fifteen cm clay pots were filled with 1 kg of such soil-compost mixture and then these pots were autoclaved and used for all the studies.

Surface sterilized seeds of tomato were sown in a

big pot containing autoclaved soil-compost mixture for raising seedlings. Surface sterilization of seeds was done by treating them in 0.1% solution of mercuric chloride for 2 minutes. Then these seeds were washed with sterilized distilled water to remove any traces of mercuric chloride. Three-week old seedlings were transferred to the 15 cm autoclaved pots singly. In case of okra 4-5 surface sterilized seeds were directly sown in the pots. After emergence, the seedlings were thinned and only one seedling was allowed to grow in one pot. When three-week old, these plants were inoculated with the nematodes, as per schedule of the experiment. In case of tomato, inoculation was done 3 days after transplantation after the seedlings were properly established. The pots were placed on a green-house bench in a randomized manner. Necessary weeding and watering was done as and when required.

2.4. Inoculation schedule:

The plants of tomato and okra were raised according to the procedure described in 2.3 and inoculated with nematodes according to the following schedule:

1. Uninoculated (control)
2. *Rotylenchulus reniformis* alone (500/plant)
3. *Meloidogyne incognita* alone (50/plant)
4. " " " (500/plant)
5. " " " (5000/plant)
6. " " (50/plant) + *R. reniformis* (500/plant)
7. " " (500/plant) + " " (500/plant)
8. " " (5000/plant) + " " (500/plant).

2.5. Recording of the data:

The experiments were terminated 2 months after inoculation. Plants were uprooted and the roots were thoroughly and gently washed with running water. The recording of the data was done as under:

2.5.1. Water absorption capability of roots:

The water absorption capability of roots was determined by the method described by Alam et al. (1974).

Erlenmeyer flasks (250 ml capacity) were filled with known amount of water. The plants were kept singly in these flasks with their roots dipped in water. Flasks without plants served as control. After 24 hours the remaining quantity of water was weighed. Amount of water lost from the control flasks was taken as water lost by surface evaporation and was deducted from the amount of water lost from other flasks and thus the amount of water absorbed by the roots was determined.

2.5.2. Plant growth:

The length (in cm) and fresh/dry weights (in g) of shoot and root were taken separately. Before taking the fresh weight excess amount of water was removed by putting the roots and shoots between blotting sheets and then separately weighed.

2.5.3. Root-knot development and nematode population in soil:

The degree of root infection caused by the root-knot nematode was assessed according to the rating scale of Tylor & Sasser (1978) as under:

Gall index (GI) or Eggmass index (EI)	Number of galls or Eggmasses
0	0
1	1 - 2
2	3 - 10
3	11 - 30
4	31 - 100
5	> 100

In the case of the reniform nematode and the root-knot larvae the soil from each treatment was processed after the termination of the experiment according to Cobb's sieving and decanting method following by the modified Baermann funnel technique (Southey, 1970). The population of the nematodes in the soil per pot was determined as per procedure described in 2.2. Reproduction factor (R) of the nematodes was calculated by the formula

of Oostenbrink (1966) as follows:

$$R = Pf/Pi$$

(where, Pf represents the final population and Pi represents the initial population of the nematodes).

2.5.4. Statistical analysis:

Statistical analysis of the data for critical difference (C.D.) at P = 0.05 and P = 0.01 levels was done as per procedure described by Pansey & Sukhatme (1978).

RESULTS

3. RESULTS

3.1. Effect of the root-knot nematode, Meloidogyne incognita and the reniform nematode, Rotylenchulus reniformis singly or concomitantly on the plant growth, root-knot development and their final populations on tomato cv. 'Pusa Ruby':

The shoot lengths of tomato plants in pots inoculated with Rotylenchulus reniformis alone (500 per pot), Meloidogyne incognita alone (50), M. incognita alone (500), M. incognita alone (5000), M. incognita (50) + R. reniformis (500), M. incognita (500) + R. reniformis (500), M. incognita (5000) + R. reniformis (500) were 36.83, 37.66, 34.66, 30.80, 32.50, 32.00 and 30.00 cm respectively as against 38.66 cm in uninoculated control (Table 1). The root lengths of the plants in the above treatments were 19.83, 21.00, 19.00, 17.00, 21.66, 18.66 and 16.50 cm as against 25.00 cm in uninoculated control (Table 1). The corresponding figures for total length of the plants in the above treatments were 56.66, 58.66, 53.66, 47.80, 54.16, 50.66 and 46.50 cm respectively as against 63.66 cm in uninoculated control (Table 1).

The shoot weights of the plants in the above treatments were 20.83, 22.50, 17.33, 16.00, 19.10, 17.00 and 16.33 g respectively as against 24.66 g in uninoculated control (Table 1). The root weights were 6.83, 9.00, 6.43,

Table 1 : Effect of the root-knot nematode, *Meloidogyne incognita* and the reniform nematode, *Rotylenchulus reniformis* singly or concomitantly on the plant growth, root-knot development and their final populations on tomato cv. 'Pusa Ruby' (Values are mean of 3 replicates).

Inoculation schedule	Plant length (cm)			Plant weight (g)			Root-knot index	Final Population of nematode			
	Shoot	Root	Total	Shoot	Root	Total		*M. incognita*		*R. reniformis*	
								Pop.	R	Pop.	R
Uninoculated (control)	38.66	25.00	63.66	24.66	15.66	40.32	-				
R. reniformis alone (500)	36.83	19.83	56.66	20.83	6.83	27.66	-			945	1.890
M. incognita alone (50)	37.66	21.00	58.66	22.50	9.00	31.50	0.70	710	14.200		
" " " (500)	34.66	19.00	53.66	17.33	6.43	23.76	1.58	1453	2.906		
" " " (5000)	30.80	17.00	47.80	16.00	4.66	20.66	4.00	8742	1.748		
" " (50) + *R. reniformis* (500)	32.50	21.66	54.16	19.10	8.10	27.20	0.56	673	13.460	842	1.684
" " (500) + *R. reniformis* (500)	32.00	18.66	50.66	17.00	5.43	22.43	1.25	1273	2.546	747	1.494
" " (5000) + *R. reniformis* (500)	30.00	16.50	46.30	16.33	3.53	19.86	4.00	7856	1.571	665	1.330
C.D. (P = 0.05)			5.97			4.51	0.71				
C.D. (P = 0.01)			8.28			6.26	1.01				

Reproduction factor (R) = Final population (Pf)/Initial population (Pi).

4.66, 8.10, 5.43 and 3.53 g respectively as against 15.66 g in uninoculated control (Table 1). The corresponding weights of the plants in the above treatments were 27.66, 31.50, 23.76, 20.66, 27.20, 22.43, and 19.86 g as against 40.32 g in uninoculated control (Table 1).

The root-knot indices were 0.70, 1.58, 4.00, 0.56, 1.25, and 4.00 respectively in plants inoculated with M. incognita alone (50 per pot), M. incognita alone (500), M. incognita alone (5000), M. incognita (50) + R. reniformis (500), M. incognita (500) + R. reniformis (500), and M. incognita (5000) + R. reniformis (500) (Table 1).

The corresponding figures for the population of M. incognita juveniles in soil and roots were 710, 1453, 8742, 673, 1273, and 7856 per pot and for the reproduction factor (R) 14.200, 2.906, 1.748, 13.460, 2.546, and 1.571 (Table 1). While the soil populations of R. reniformis around plants inoculated with R. reniformis alone (500 per pot), M. incognita (50) + R. reniformis (500), M. incognita (500) + R. reniformis (500), and M. incognita (5000) + R. reniformis (500) were 945, 842, 747, and 665 respectively, with reproduction factor 1.890, 1.684, 1.494, and 1.330 respectively (Table 1).

3.2. Effect of the root-knot nematode, Meloidogyne incognita and the reniform nematode, Rotylenchulus reniformis singly or concomitantly on the water absorption capability of roots of

tomato cv. 'Pusa Ruby':

The amounts of water absorbed per plant when inoculated with R. reniformis alone (500 per pot), M. incognita alone (50), M. incognita alone (500), M. incognita alone (5000), M. incognita (50) + R. reniformis (500), M. incognita (500) + R. reniformis (500), and M. incognita (5000) + R. reniformis (500) were 40.00, 39.00, 32.00, 27.80, 34.30, 29.00, and 24.70 g respectively as against 44.16 g in uninoculated control (Table 2).

3.3. Effect of the root-knot nematode, Meloidogyne incognita and the reniform nematode, Rotylenchulus reniformis singly or concomitantly on the plant growth, root-knot development and their final populations on okra cv. 'Pusa Sawani':

The shoot lengths of the okra plants inoculated with R. reniformis alone (500 per pot), M. incognita alone (50), M. incognita alone (500), M. incognita alone (5000), M. incognita (50) + R. reniformis (500), M. incognita (500) + R. reniformis (500), and M. incognita (5000) + R. reniformis (500) were 30.50, 36.50, 32.20, 29.25, 31.16, 30.16, and 28.50 cm respectively as against 34.36 cm in uninoculated control (Table 3). The root lengths of the above plants were 28.50, 25.75, 20.60, 17.25, 24.43, 19.84, and 16.10 cm as against 35.26 cm in uninoculated control (Table 3). The total lengths of the plants were 59.00, 62.25, 52.80, 46.50,

Table 2 : Effect of the root-knot nematode, *Meloidogyne incognita* and the reniform nematode, *Rotylenchulus reniformis* singly or concomitantly on the water absorption capability of roots of tomato c v. 'Pusa Ruby' (Values are mean of 3 replicates).

Inoculation schedule	Amount of water absorbed per plant (g)
Uninoculated (control)	44.16
R. reniformis alone (500)	40.00
M. incognita " (50)	39.00
" " (500)	32.00
" " (5000)	27.80
" " (50) + *R. reniformis* (500)	34.30
" " (500) + " " (500)	29.00
" " (5000) + " " (500)	24.70
C.D. (P = 0.05)	3.20
C.D. (P = 0.01)	4.44

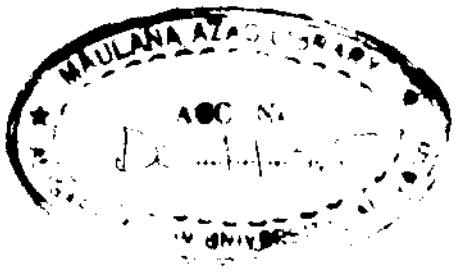

Table 3 : Effect of the root-knot nematode, Meloidogyne incognita and the reniform nematode, Rotylenchulus reniformis singly or concomitantly on the plant growth, root-knot development and their final populations on okra cv. 'Pusa sawani' (Values are mean of 3 replicates).

Inoculation schedule	Plant length (cm)			Plant weight (g)			Root-knot index	Final population of nematode			
	Shoot	Root	Total	Shoot	Root	Total		M. incognita		R. reniformis	
								Pop.	R	Pop.	R
Uninoculated (control)	34.36	35.26	69.62	8.33	3.76	12.09	-				
R. reniformis alone (500)	30.50	28.50	59.00	6.00	2.90	8.90	-			925	1.850
M. incognita alone (50)	36.50	25.75	62.25	7.75	3.75	11.50	0.60	821	16.420		
" " " (500)	32.20	20.60	52.80	5.40	2.80	8.20	1.50	1480	2.960		
" " " (5000)	29.25	17.25	46.50	4.35	2.10	6.45	4.00	8800	1.760		
" " (50) + R. reniformis (500)	31.16	24.43	55.59	6.00	2.70	8.30	0.50	710	14.200	880	1.760
" " (500) + R. reniformis (500)	30.16	19.84	50.00	4.60	2.36	6.96	1.25	1330	2.660	789	1.578
" " (5000) + R. reniformis (500)	28.50	16.10	44.60	4.10	1.95	6.05	4.00	8000	1.600	598	1.196
C.D. (P = 0.05)			7.18			1.47	0.43				
C.D. (P = 0.01)			9.97			2.05	0.61				

Reproduction factor (R) = Final population (Pf)/Initial population (Pi).

55.59, 50.00, and 44.60 cm respectively as against 69.62 cm in uninoculated control (Table 3).

The corresponding shoot weights of plants in the above treatments were 6.00, 7.75, 5.40, 4.35, 6.00, 4.60, and 4.10 g respectively as against 8.33 g in uninoculated control (Table 3). The root weights were 2.90, 3.75, 2.80, 2.10, 2.70, 2.36, and 1.95 g as against 3.76 g in uninoculated control (Table 3). The corresponding weights of the plants in the above treatments were 8.90, 11.50, 8.20, 6.45, 8.30, 6.96, and 6.05 g as against 12.09 g in uninoculated control (Table 3).

The root-knot indices were 0.60, 1.50, 4.00, 0.50, 1.25, and 4.00, respectively in plants inoculated with M. incognita alone (50 per pot), M. incognita alone (500), M. incognita alone (5000), M. incognita (50) + R. reniformis (500). M. incognita (500) + R. reniformis (500), and M. incognita (5000) + R. reniformis (500) respectively (Table 3).

The corresponding figures for the populations of 2-nd stage juveniles of M.incognita in soil and roots were 821, 1480, 8800, 710, 1330, and 8000 per pot and for the reproduction factor (R) 16.420, 2.960, 1.760, 14.200, 2.660, and 1.600 (Table 3). While the soil populations of R. reniformis around plants inoculated with R. reniformis

alone (500 per pot), M. incognita (50) + R. reniformis (500), M. incognita (500) + R. reniformis (500), and M. incognita (5000) + R. reniformis (500) were 925, 880, 789, and 598 respectively, with reproduction factor 1.850, 1.760, 1.578, and 1.196 respectively (Table 3).

3.4. Effect of the root-knot nematode, Meloidogyne incognita and the reniform nematode, Rotylenchulus reniformis singly or concomitantly on the water absorption capability of roots of okra cv. 'Pusa Sawani':

The amounts of water absorbed per plant when inoculated with R. reniformis alone (500 per pot), M. incognita alone (50), M. incognita alone (500), M. incognita alone (5000), M. incognita (50) + R. reniformis (500), M. incognita (500)+R. reniformis (500), and M. incognita (5000) + R. reniformis (500) were 14.00, 14.50, 13.50, 12.50, 14.00, 13.00, and 12.33 g respectively as against 15.50 g in uninoculated control (Table 4).

Table 4 : Effect of the root-knot nematode, Meloidogyne incognita and the reniform nematode, Rotylenchulus reniformis singly or concomitantly on the water absorption capability of roots of okra cv. 'Pusa Sawani' (Values are mean of 3 replicates).

Inoculation schedule	Amount of water absorbed per plant (g)
Uninoculated (control)	15.50
R. reniformis alone (500)	14.00
M. incognita alone (50)	14.50
" " " (500)	13.50
" " " (5000)	12.50
" " (50) + R. reniformis (500)	14.00
" " (500) + " " (500)	13.00
" " (5000) + " " (500)	12.33
C.D. (P = 0.05)	1.78
C.D. (P = 0.01)	2.47

DISCUSSION

4. DISCUSSION

Both the test nematodes multiplied freely on tomato cv. 'Pusa Ruby' and okra cv. 'Pusa Sawani'. The reproduction factor (R) of Rotylenchulus reniformis was 1.89 and 1.85 on tomato and okra respectively. In case of Meloidogyne incognita the R factor was 14.20 and 16.42 respectively on tomato and okra when initial inoculum level was 50 larve per pot. The corresponding figure for higher inoculum levels were 2.906 and 2.960 at 500 inoculum level, and 1.748 and 1.760 at 5000 inoculum level. This is understandable because the multiplication rate depends mainly on the availability of food; higher the inoculum level more is the competition for the food (Tables 1,3).

However, in concomitant inoculations of the test nematodes an antagonistic relationship was noted in both the crops as indicated by reduced R factor of both the nematodes as well as root-knot development caused by the root-knot nematode. Similar antagonistic relationship between M. incognita and R. reniformis has also been observed by Singh (1976) on soybean, Misra & Gaur (1981) on black gram, Misra & Gaur (1981) on Moth bean, Ras & Seshadri (1981) on grape, and Thomas & Clark (1980, 1981, 1983) on sweet potato.

The plant growth characters were found inversely correlated with the level of inoculum of both the nematode species. The test nematodes individually caused significant

reduction in plant growth but the effect was more pronounced on fresh weight than the plant length. A moderate inoculum density of M. incognita (500 larvae/plant) produced relatively greater decrease in plant growth than R. reniformis at the same inoculum level (Tables 1,3).

In both the crops the reduction in plant growth was more when both the nematodes were present concomitantly. Therefore they exhibited a synergistic effect (Tables 1,3). Our results are in confirmity with those obtained by Singh (1976) on Soybean, Misra & Gaur (1981) on black gram, Misra & Gaur (1981) on moth bean, Ras & Seshadri (1981) on grape, and Thomas & Clark (1980,1981,1983) on sweet potato.

The water absorption capability of tomato and okra roots was found inversely correlated with the level of inoculum of both the nematodes (Tables 2,4). The inhibition in water absorption was, however, more when both the nematodes were inoculated concomitantly, thereby showing a synergistic effect.

The present study has confirmed and extended the findings of Alam et al., (1974) and Alam & Ismail (1975) who have also noted inhibition in water absorption capability of tomato roots due to the infection by Meloidogyne incognita. As suggested by them, the inhibition in water absorption in the present case may be due to deformation, choking and

disturbance in the arrangement of tracheary elements. Endo (1971,1975) reported various abnormalities in tissues due to hyperplastic and hypertrophic arrangement of cells in root-knot nematode infected roots. Moreover, Swami & Krishnamurthy (1971) have observed mechanical damage of cells, disruption in the arrangement of tracheary elements, deformation and blockage of vessels. Probably these abnormalities are responsible for the poor water absorption. The root-knot infection brought about reduction in root weights (Table 1,3) or in other words total surface area of roots. This may be the other reason. Moreover, the shoot weight was also reduced which might have caused a check in the transpiration pull and which might be indirectly responsible for the poor absorption of water.

In case of R. reniformis, the present findings are similar to those of Ismail & Alam (1975) who have also reported inhibitary effect of the nematode on water absorption capability of tomato and castor. Poor root growth, reduced transpiration pull and histological aberrations (Endo,1975) caused by R. reniformis, might be the reasons for the poor water absorption by the roots.

Tiyagi et al. (1986) have also found that the water absorption by chickpea roots was retarded due to the infection of M. incognita and R. reniformis.

SUMMARY

5. SUMMARY

The root-knot nematode, Meloidogyne incognita and the reniform nematode, Rotylenchulus reniformis caused significant reduction in plant growth of tomato cv. 'Pusa Ruby' and okra cv. 'Pusa Sawani'. Consequently water absorption capability of roots was impaired. In concomitant infections the reduction in plant growth as well as in water absorption capability of roots was more pronounced. Thus a synergistic phenomenon was noted. In single as well as in combined inoculations the reduction in plant growth and in water absorption capability of roots was found directly proportional to the inoculum level of both the nematodes.

Both the test plants were found good hosts for the test nematodes as they supported their population build-up. However, in co-habitation the nematodes were antagonistic to each other. The population build-up of M. incognita was greater at lower inoculum level than higher inoculum levels.

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